

# Amniotic fluid insulin concentration as a predictor of obesity

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## Abstract

Longitudinal correlations were obtained between amniotic fluid insulin concentration at 32 to 38 weeks' gestation and anthropometric characteristics at the age of 6 years in 56 children of diabetic mothers. The prospective studies indicated that at the age of 6 years, as at birth, the greatest increase in weight in relation to height (relative obesity) was seen in children who experienced the greatest exposures to insulin in the uterus (as judged by amniotic fluid insulin concentration). Significant correlations between amniotic fluid insulin and relative obesity at the age of 6 years were found after adjustment for maternal obesity and macrosomia at birth. The highest amniotic fluid insulin values are clustered in the subgroup of 14 children who were obviously obese by the age of 6 years. These findings are consistent with the hypothesis that there is an association between anthropometric development and intrauterine metabolism, and suggest that premature and excessive exposure to insulin during gestation may predispose to obesity in childhood. The amniotic fluid insulin concentration may predict this eventuality.

The possibility that maternal metabolism may exert an influence on the development of children was suggested by Freinkel a decade ago and designated as 'fuel-mediated teratogenesis'.<sup>1,2</sup> He proposed that maternal fuels may influence developmental events by modifying phenotypic gene expression in terminally differentiated, poorly replicating cells during intrauterine development; in addition, the long range effects will depend on which cells undergo differentiation, proliferation, or functional maturation (or a combination) at the time of the disturbance in maternal metabolism.<sup>1,2</sup>

The Diabetes in Pregnancy Center was established at Northwestern University Medical School in 1977 to test this hypothesis prospectively. Diabetes was chosen as the disease for study because maternal fuels undergo the greatest changes in this condition. Pregnant women with pregestational as well as gestational diabetes mellitus were enrolled during the period 1977-83. Their fuel economy was carefully monitored throughout pregnancy, and we have been following up their children since then in a prospective attempt to correlate their long range development with maternal metabolism during pregnancy. The present study reports some of the anthropometric consequences of intrauterine metabolic experiences ('fuel-related anthropometric teratogenesis'). We found significant correlations between pancreatic islet function of the fetus within the uterus and body shape in childhood. These conclusions

are based on measurements in 56 children who now have reached the age of 6 years and in whom estimates of amniotic fluid insulin were obtained during weeks 32-38 of pregnancy. As far as we know this is the first direct evidence that exposure of a fetus to insulin may serve as a prognostic indicator of obesity in childhood.

## Subjects and methods

Fifty six mothers were enrolled into this prospective study during the period 1977-83. The group was composed of 26 women with known diabetes mellitus who were receiving treatment before the index pregnancy (pregestational diabetes mellitus) and 30 with gestational diabetes mellitus. All the subjects with pregestational diabetes mellitus and 18 of 30 with gestational diabetes mellitus were treated with insulin given subcutaneously, as soluble insulin before each major meal (three times a day) and as intermediate acting insulin once or twice daily to sustain basal needs. Our objectives were to achieve 'tight' glycaemic control without frequent or severe attacks of hypoglycaemia, and to defer delivery until full term had been reached. Metabolic control was assessed from daily records of glucosuria and acetonaemia, weekly or twice weekly measurements of fasting plasma glucose concentration, and monthly measurements of glycated haemoglobin (HbA<sub>1c</sub>).

Amniotic fluid was sampled to monitor fetal lung maturation, and aliquots were frozen for later measurements of immunoreactive insulin concentrations.<sup>3</sup> Amniocentesis was carried out every two weeks starting at 32-34 weeks' gestation until full term. We found no association between insulin concentration and gestational age during this period. When two or more measurements were available the mean value was used. Anthropometric assessments were made of the infants at birth, and during subsequent follow up examinations by a variant of a measurement first proposed by Farquhar that we have designated the symmetry index.<sup>4</sup> On each occasion relative weight (that is, observed weight in relation to median weight for that age) to relative height (that is, observed height in relation to median height for that age) as follows:

$$\text{Symmetry index} = \frac{\text{observed weight/median for age}}{\text{observed height/median for age}}$$

According to this calculation a symmetry index of 1.0 indicates symmetrical adipose tissue and skeletal growth, whereas values of over 1.0 reflect asymmetry with relatively disproportionate increases in fat. On the basis of our experience with normal newborn infants and young children, symmetry indexes of over 1.2 indicate obesity. We

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have ascribed such asymmetrical relationships during periods of active growth to disproportionately greater development in structures that are most sensitive to the action of insulin than in those that are relatively insensitive.<sup>1 5</sup>

Analysis of variance and the Duncan method<sup>6</sup> were used to assess the significance of differences among the groups.

### Results

Mean (SEM) gestational age at delivery in the 56 infants of diabetic mothers was 38.5 (0.2) weeks. At birth, their mean (SEM) weight was 3653 (87) g (range 2200–5580); length (height) 51.0 (0.4) cm (range 46.0–59.0); and symmetry index 1.17 (0.02) (range 0.88–1.53).

Mean estimates of immunoreactive insulin concentrations in amniotic fluid taken during weeks 32–38 of pregnancy correlated significantly with symmetry index at birth after controlling for maternal obesity by covariate analysis ( $r=0.362$ ;  $p=0.003$ ). The relationship was not, however, a simple linear one. Figures 1 and 2 show the antepartum amniotic fluid insulin concentrations and symmetry index values measured at the time of birth and at the age of 6 years in the 56 infants. Based on the symmetry index values these subjects were assigned to one of the three groups (group 1: symmetry index  $<1.0$ , group 2: symmetry index  $1.0-1.2$ , and group 3: symmetry index  $>1.2$ ). At birth (fig 1) seven (12%), 28 (50%), and 21 (38%) subjects were in groups 1, 2, and 3 respectively. There were significant differences among the groups (analysis of variance,  $p<0.05$ ). Moreover, the amniotic fluid insulin concentration was significantly higher in group 3 (symmetry index  $>1.2$ ) than group 2 (symmetry index  $1.0-1.2$ ), mean (SD) values being 125.5 (86.1) compared with 72.3 (62.8) pmol/l ( $p<0.05$ ).

At the age of 6, the height of the subjects did not differ from that expected for their age (observed height/median height for age = 1.006 (0.039)). By contrast, mean weight was substantially increased (observed weight/median weight for age = 1.137 (0.220)). The symmetry index for the whole group was 1.126 (0.185). When the infants were divided into three groups according to their symmetry index at the age of 6 years, a similar distribution of amniotic fluid insulin concentrations was observed (fig 2). A significant correlation between antepartum amniotic fluid insulin concentration and sym-

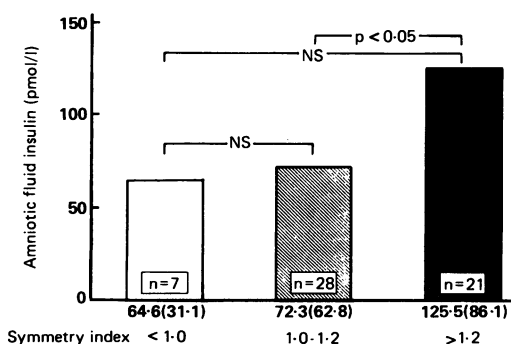


Figure 1 Association between symmetry index at birth in 56 children of diabetic mothers and amniotic fluid insulin concentrations during late gestation. The bars denote the mean amniotic fluid insulin concentration for each group; the values under each bar are the mean (SD). Significance of the differences between groups was determined by the Duncan method.<sup>6</sup>

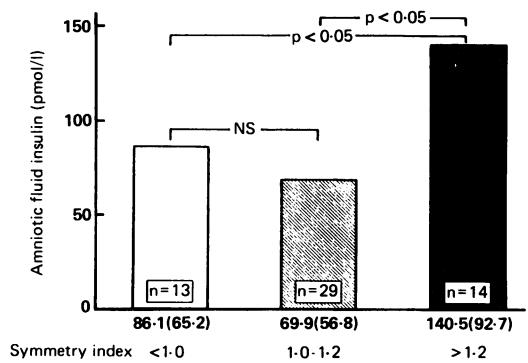


Figure 2 Association between symmetry index at age 6 and amniotic fluid insulin concentration during late gestation. The bars denote the mean amniotic fluid insulin concentration for each group; the values under each bar are mean (SD). Significance of the differences between groups was determined by the Duncan method.<sup>6</sup>

metry index values at the age of 6 years was seen in the entire group, even when we controlled for maternal obesity and symmetry index at birth as covariables ( $r=0.262$ ;  $p=0.03$ ). There were significant differences among the three groups (analysis of variance,  $p<0.01$ ). On further analysis the highest amniotic fluid insulin concentrations that we had observed before birth were clustered predominantly and significantly in that subgroup of 14 infants (25%) with obvious obesity at the age of 6 years—that is, group 3 who had symmetry index values of over 1.2. Eight of these 14 infants had also had symmetry indexes of  $>1.2$  at birth. On further analysis by the Duncan method,<sup>6</sup> antepartum amniotic fluid insulin concentrations in group 3 (symmetry index  $>1.2$  at the age of 6) were significantly higher ( $p<0.05$ ) than in the other two groups (140.5 (92.7), 86.1 (65.2), and 69.9 (56.8) pmol/l respectively).

### Discussion

During normal intrauterine life maternal insulin does not cross the placenta,<sup>7 8</sup> the secretion of fetal insulin is limited, and the functional responsiveness of fetal islets to nutrient secretagogues is tardy.<sup>9-11</sup> Hyperplasia, hypertrophy, and heightened functional reactivity supervene in fetal islets in pregnancies that are complicated by diabetes, presumably in response to the increased transplacental delivery of glucose<sup>12</sup> and other nutrient secretagogues.<sup>1 13</sup> Accordingly, late fetal development in an infant of a mother with diabetes may occur in a relatively insulin enriched environment. There are no easy ways of sampling fetal insulin production directly; because amniotic fluid insulin is wholly derived from fetal islets,<sup>14</sup> however, the concentration of insulin in amniotic fluid may act as an inferential index of integrated insulin secretion by the fetus, despite the uncertainties concerning rates of turnover and variations in the volume of amniotic fluid. We<sup>5 15</sup> and others<sup>16 17</sup> have previously shown that amniotic fluid insulin concentrations may be increased in even the mildest forms of gestational diabetes mellitus thus illustrating the exquisite sensitivity of fetal islet development to the stimulatory effects of ambient nutrients. As shown by symmetry index values at birth,<sup>18</sup> and patterns of intrauterine growth during serial ultrasonography,<sup>5 19</sup> the fetus of the diabetic mother seems to develop in an asymmetrical fashion whenever fetal insulin is increased so that insulin sensitive structures are stimulated to a dis-

proportionate degree; this asymmetry seems to be characteristic of the macrosomia that has long been recognised in the offspring of diabetic mothers. The experiments of Susa *et al* with insulin mini-pumps implanted in the fetuses of non-diabetic, normoglycemic primates suggest that such asymmetrical development within the uterus can occur even without increases in the concentrations of circulating fuels and may be driven in a large part, by the premature, and perhaps inappropriate, availability of 'extra' insulin.<sup>20</sup>

The present findings assume particular importance in the above context. As judged by amniotic fluid insulin, we have shown that an increased availability of insulin to the fetus of the diabetic mother during intrauterine development may correlate not only with anthropometric characteristics at birth, but also with subsequent patterns of asymmetrical development—that is, the development of childhood obesity by the age of 6. Analysis of our follow up data indicates that this childhood obesity may be dissociated from maternal obesity and that it may supervene even when symmetry index values are less than 1.2 at birth. It is tempting to suggest that some of this propensity for asymmetrical growth and childhood obesity may be linked to the developmental impact of the accelerated islet maturation and enhanced insulin secretion during fetal life. The increases in amniotic fluid insulin could thus provide some direct augury for the long range consequences of such upsets of the developmental timetables ('fuel-mediated anthropometric teratogenesis').

While our prospective studies have been underway, Pettitt *et al* have reported their findings with the Pima Indians.<sup>21</sup> Review of the longitudinal data from this highly inbred population who are prone to non-insulin dependent diabetes mellitus has indicated that the infants of mothers who were diabetic during pregnancy already had a far greater incidence of obesity by the ages of 5–9 than those of mothers who became diabetic after the index pregnancy or remained normal, and that the distinctions were even more pronounced by the ages of 10–14 and 15–19.<sup>21</sup> Moreover, in the Pima Indians—as in the present series—the putative correlations between childhood or adolescent obesity and metabolic experiences during fetal life could also be disassociated from obesity in the mother, or the occurrence of macrosomia at birth, or both.<sup>21, 22</sup> Unfortunately the available information concerning the state of maternal metabolism throughout pregnancy, and the attendant implications for the developing fetuses in the studies of Pima Indians, is limited to the single measurement of maternal plasma glucose that was made two hours after a glucose load had been given to classify gestational glucose tolerance. None the less, these important observations also implicate the maternal environment as a contributor to subsequent obesity and are consistent with the hypothesis of 'fuel-mediated anthropometric teratogenesis'.<sup>1, 2</sup>

Our prospective study has shown that amniotic fluid insulin concentration as an index of prematurely activated fetal insulin secretion may be a prognostic indicator of obesity in childhood. Whether the premature exposure of the fetus to insulin affects subsequent anthropometrics by phenotypic modifications of cells concerned in

postnatal sensitivity to insulin in the periphery, insulin secretion, or neuroendocrine factors that govern energy expenditure or fuel intake, or counterregulation, or a combination, are questions that we are investigating in our continuing studies.

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